Ch14 Picornaviruses (and other plus-strand RNA viruses)

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Family *Picornaviridae*

Pico (= small) **RNA** viruses

Hosts: mammals
birds

Diseases: common cold
polio
hepatitis A
foot and mouth disease

Virion

- icosahedral
- 25–30 nm diameter
- genome: single-stranded RNA
  - Plus polarity
  - 7–8 kb
  - Covalently linked protein (VPg)

The virus genome can function as mRNA.

At a glance

14.1 Introduction to picornaviruses (小RNA病毒)

- Members of the family *Picornaviridae* are found in mammals and birds.

Genus	Name derivation from Greek word	Example(s)
Hepatovirus 肝病毒屬	*Hepatos* = liver	Hepatitis A virus A型肝炎病毒
Enterovirus 腸病毒屬	*Enteron* = intestine	Poliovirus 小兒麻痺病毒
		Coxsackieviruses 克沙奇病毒
Rhinovirus	*Rhinos* = nose	Common cold viruses
Aphthovirus 鼻病毒屬 鵝口瘡病毒屬	*Aphtha* = vesicles in the mouth	Foot and mouth disease virus 口蹄疫病毒

- Poliovirus was one of the first viruses to be propagated in cell culture (in 1949) and was also one of the first to be plaque purified (in 1954).
- Most picornaviruses grow readily in cell culture, are easy to purify and are stable, making them popular viruses for laboratory studies.
- The picornaviruses are class IV viruses.
- The first virus molecules to be synthesized in an infected cell are therefore proteins, in contrast to all other viruses, where transcription must occur before protein synthesis.

14.2 Some important picornaviruses

14.2.1 Hepatitis A virus (A型肝炎病毒)

- Genus: *Hepatovirus*
- Hepatitis A is especially prevalent in developing countries with poor sanitation.
- In most infants and young children infection is asymptomatic or mild, and leads to life-long immunity. When adults become infected, about 75% develop jaundice (黄疸); severe hepatitis is a rare complication, which can be fatal.

14.2.2 Poliovirus (小兒麻痺病毒)

- Genus: *Enterovirus*
- Poliovirus has been the subject of much research effort because of the devastating paralysis that it can cause.
  - ✓ In fact, the majority of poliovirus infections are relatively harmless infections of the oropharynx and the gut.
  - ✓ Serious disease occurs only after other tissues become infected, resulting in viraemia (virus in the blood) and spread of infection to the central nervous system.

- This is a very rare event in babies, who still have anti-poliovirus antibodies acquired from their mothers. If there is less poliovirus in the human environment, so that most infections occur after these antibodies have disappeared, then infection of the central nervous system is more likely.
- Poliovirus infection of the central nervous system may result in meningitis (from which most patients recover completely), encephalitis and/or paralytic poliomyelitis. The latter is due to virus replication in motor neurones of the spinal cord or the brain stem, resulting in paralysis of limbs and/or breathing muscles.

(a)

(b)

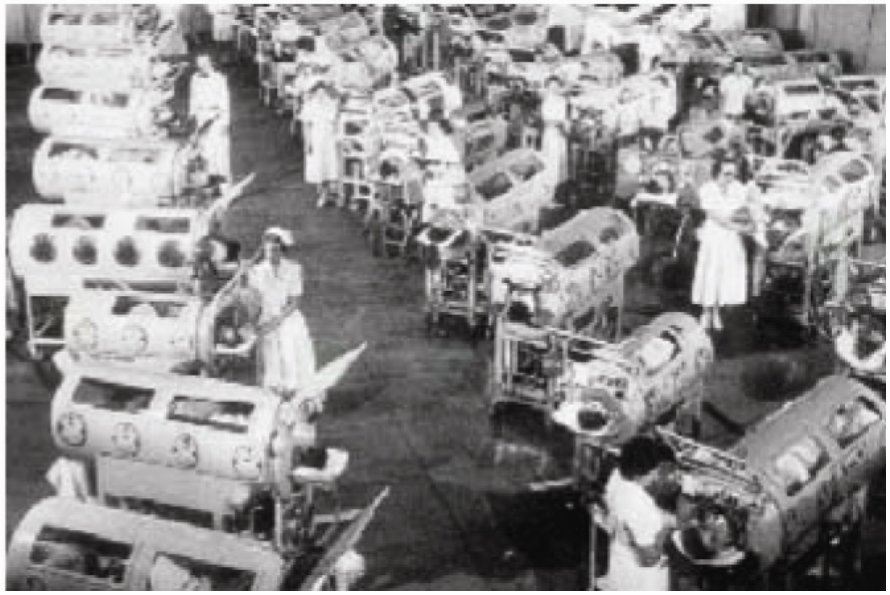


Figure 14.1 (a) *Children with paralysed limbs as a result of polio.* Reproduced by permission of the World Health Organisation. (b) Respirators ('iron lungs') used in the mid-20th century to aid the breathing of polio victims. Reproduced by permission of the US Centers for Disease Control and Prevention.

- The fear of paralysis was a major motivation for research programmes to develop polio vaccines in the mid-20th century, and these efforts led to the development of inactivated vaccines (1955 by Jonas Salk) and live attenuated vaccines (1962 by Albert Sabin).
- The use of both types of vaccine has proved very effective in preventing polio, so that by the start of the 21st century the annual number of world cases had fallen to 3500, which was 1% of the number of cases in 1988.

Derivation of attenuated poliovirus strain (Sabin type 1) from wild-type poliovirus strain (Mahoney 1)

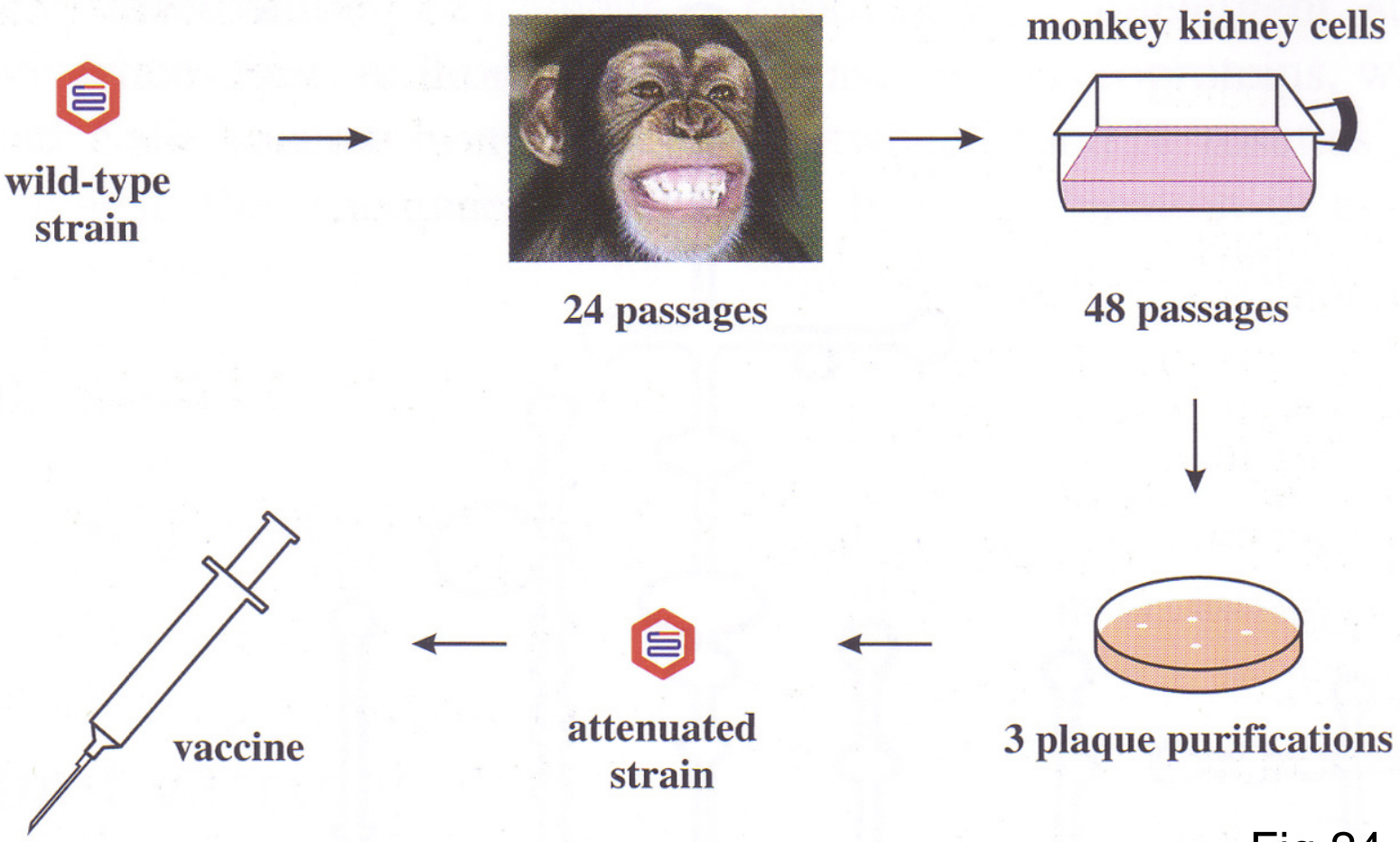
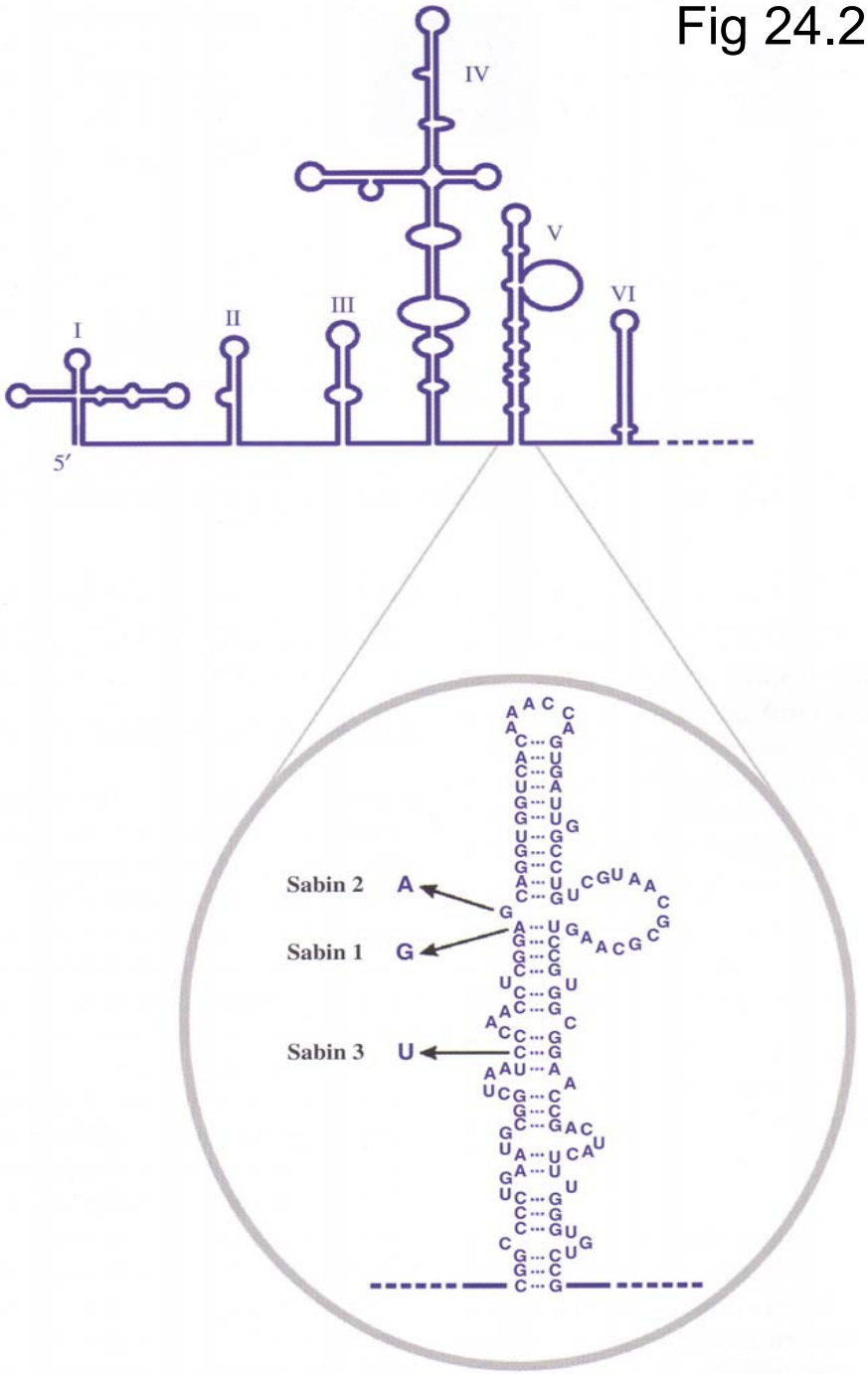


Fig 24.1

Fig 24.2



5’ end of poliovirus RNA with expanded view of domain V. For each of the three Sabin strains a mutation in domain V that contributes to the attenuation of neurovirulence is indicated.

14.2.3 Coxsackieviruses (克沙奇病毒)

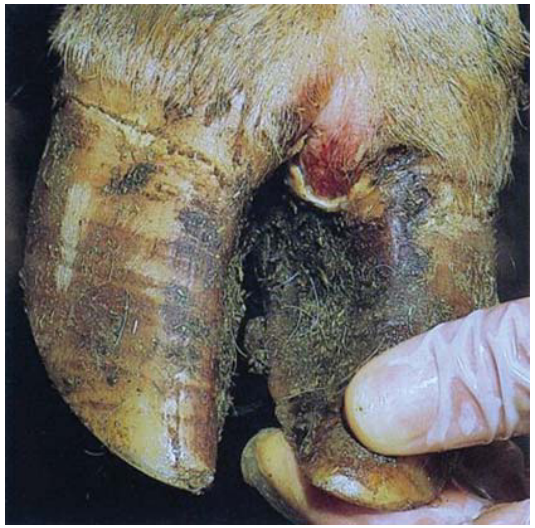
- Genus: *Enterovirus*
- In 1948, investigators in the US town of Coxsackie injected faecal specimens from two suspected polio cases into suckling mice; lesions developed in the skeletal muscles of the mice and the first coxsackieviruses had been isolated.
- Coxsackieviruses cause a range of medical conditions, including myocarditis (heart disease), meningitis and rashes.

14.2.4 Rhinoviruses (鼻病毒)

- Genus: *Rhinovirus*
- Rhinoviruses are the most common agents causing upper respiratory tract infections in humans.
- Most children have had at least one rhinovirus infection by the age of 2 years, and in adults rhinoviruses account for about 50% of common colds.
- Rhinoviruses replicate in the epithelium of the upper respiratory tract, where the temperature is around 33–35 ℃.

14.2.5 Foot and mouth disease virus (口蹄疫病毒)

- Genus: *Aphthovirus*
- Foot and mouth disease virus has a much wider host range than other picornaviruses; it infects mammals such as cattle, sheep, goats and pigs, causing lesions on the feet and in the mouth.

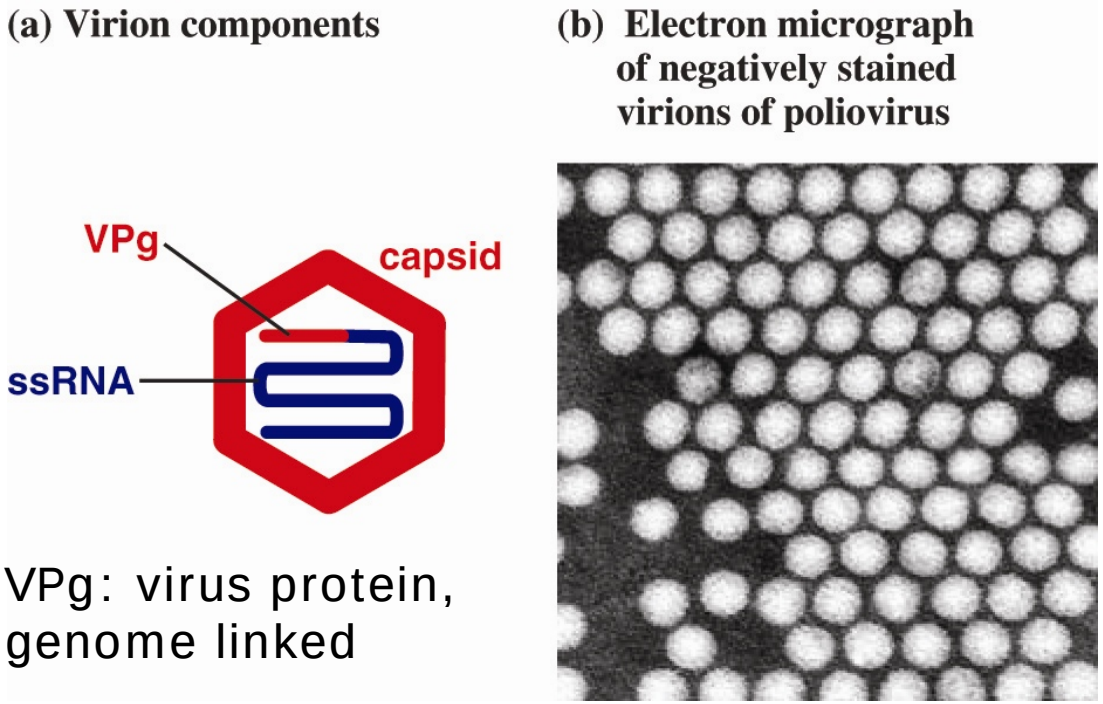


- Outbreaks of foot and mouth disease can have serious economic consequences.

- A massive outbreak in the UK in 2001 was brought under control through a series of measures that included the slaughter of more than 6 million animals.
- The estimated cost of the outbreak to the nation was over 8 billion pounds.

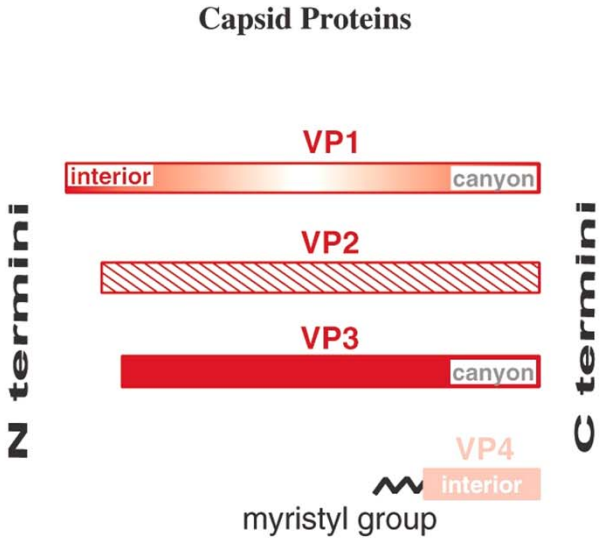
14.3 Picornavirus virion

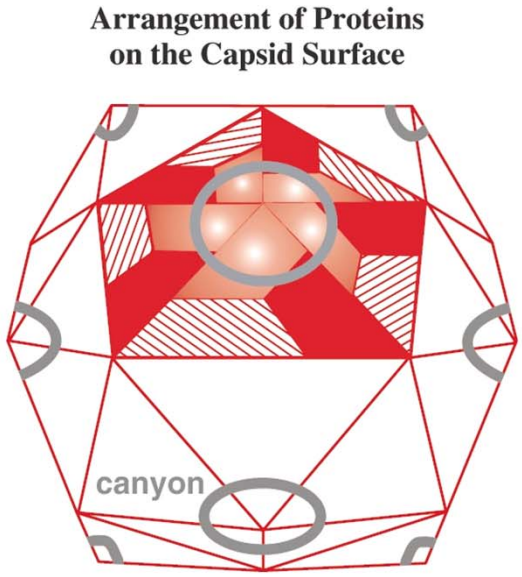
- Picornaviruses are small RNA viruses of relatively simple structure. The RNA is enclosed by a capsid, which is roughly spherical and has a diameter of about 25–30 nm.



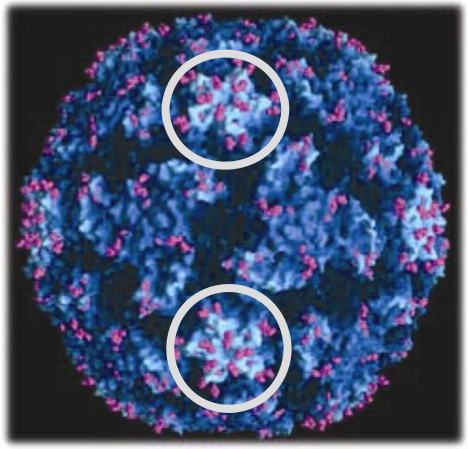
14.3.1 Capsid

- The picornavirus capsid has icosahedral symmetry and is made from 60 copies each of four virus proteins (VP1–4, numbered from the largest to the smallest).
  - ✓ parts of VP1–3 are at the surface of the virion
  - ✓ the N termini of VP1 and all 60 VP4 molecules are completely internal
- In many picornaviruses, there is a deep cleft (approximately 2 nm deep) around each of the 12 vertices of the icosahedron.





The capsid of human rhinovirus 14



Reconstructed Image from
Cryo-Electron Microscopy

- The outer surface of the capsid is composed of regions of VP1, VP2 and VP3.
- Around each of the vertices is a canyon lined with the C termini of VP1 and VP3.
- The interior surface of the capsid is composed of VP4 and the N termini of VP1.

- Evolution of picornaviruses has generated a lot of variability in the capsid proteins, some of which is reflected in the existence of distinct serotypes.

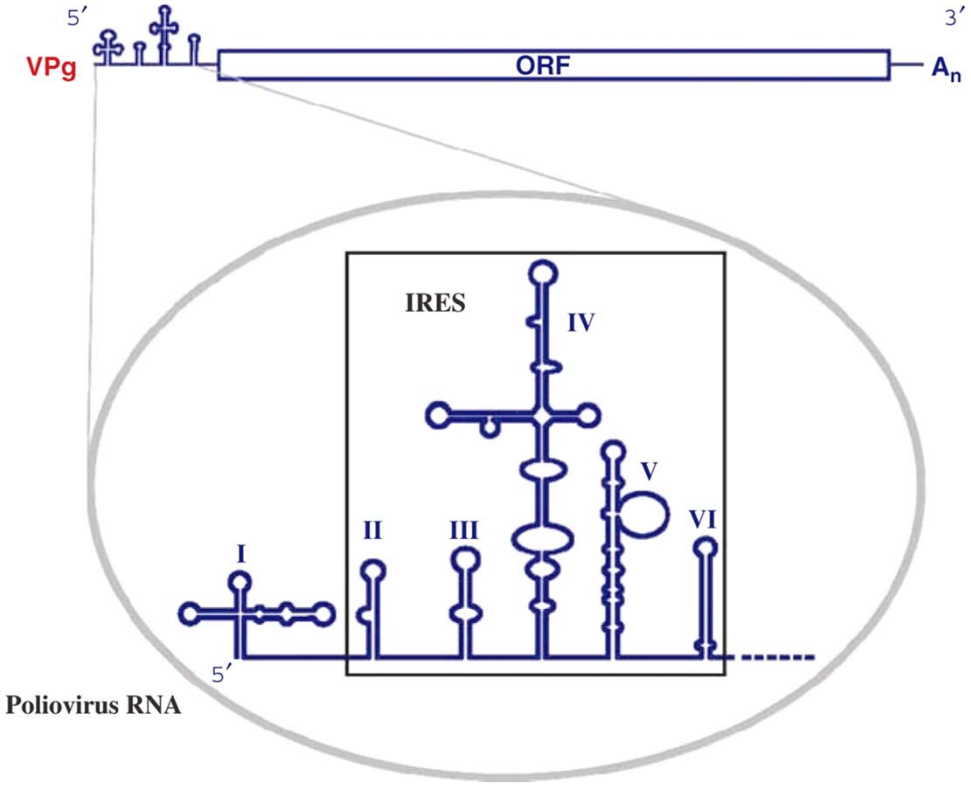
Virus	Number of serotypes
Poliovirus	3
Foot and mouth disease virus	7
Human rhinoviruses	>100

- This antigenic variability creates problems for the development of vaccines against these viruses, problems that have been overcome for poliovirus and foot and mouth disease virus, but not for the rhinoviruses.

14.3.2 Genome

- The picornavirus genome is composed of a 7–8 kb ssRNA.
- Covalently linked to the 5’ end of the RNA is a small (2–3 kD) protein known as VPg (virus protein, genome linked).
  - ✓ The covalent link is via the –OH group of a tyrosine residue at position 3 of VPg.
- The 3’ end of the RNA is polyadenylated.
- The genome consists of one very large ORF flanked by untranslated regions.

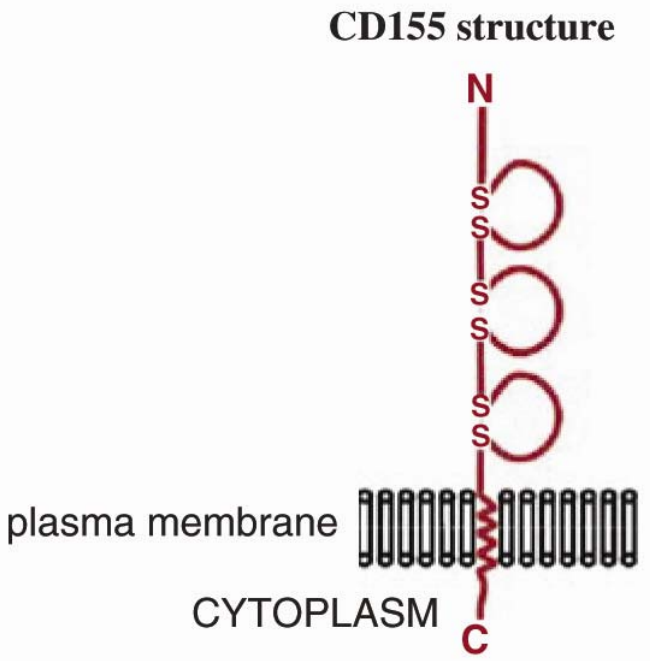
- Within the untranslated region at the 5’ end there is much secondary structure.
  - ✓ Ex: in poliovirus RNA, there are six domains, five of which form an internal ribosome entry site (IRES).



14.4 Picornavirus replication

14.4.1 Attachment

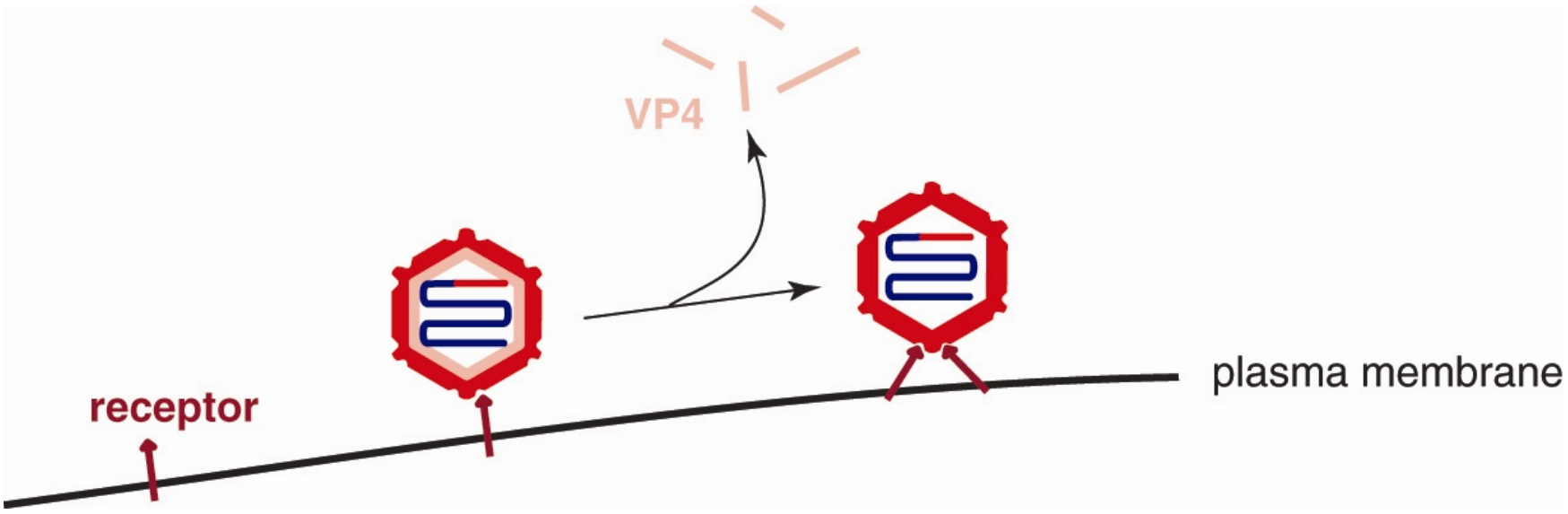
- The poliovirus receptor is the glycoprotein CD155.
  - ✓ Its function is not known, but it is expressed on many celltypes.
- CD155 is a member of the immunoglobulin superfamily of molecules with three immunoglobulin-like domains; the virus attachment site is located in the outermost domain.



The loops indicate immunoglobulin-like domains. Each domain is stabilized by one or two disulphide (-S-S-) bonds.

- For those picornaviruses like poliovirus with canyons on the virion surface, there are virus attachment sites located in pockets at the canyon bases.
  - ✓ The canyons are too narrow for access by antibodies so the virus attachment sites are protected from the host's immune surveillance, while the remainder of the virion surface can mutate to avoid the host's immune response.
- The virus attachment sites of foot and mouth disease virus are not in canyons but are on surface protrusions.

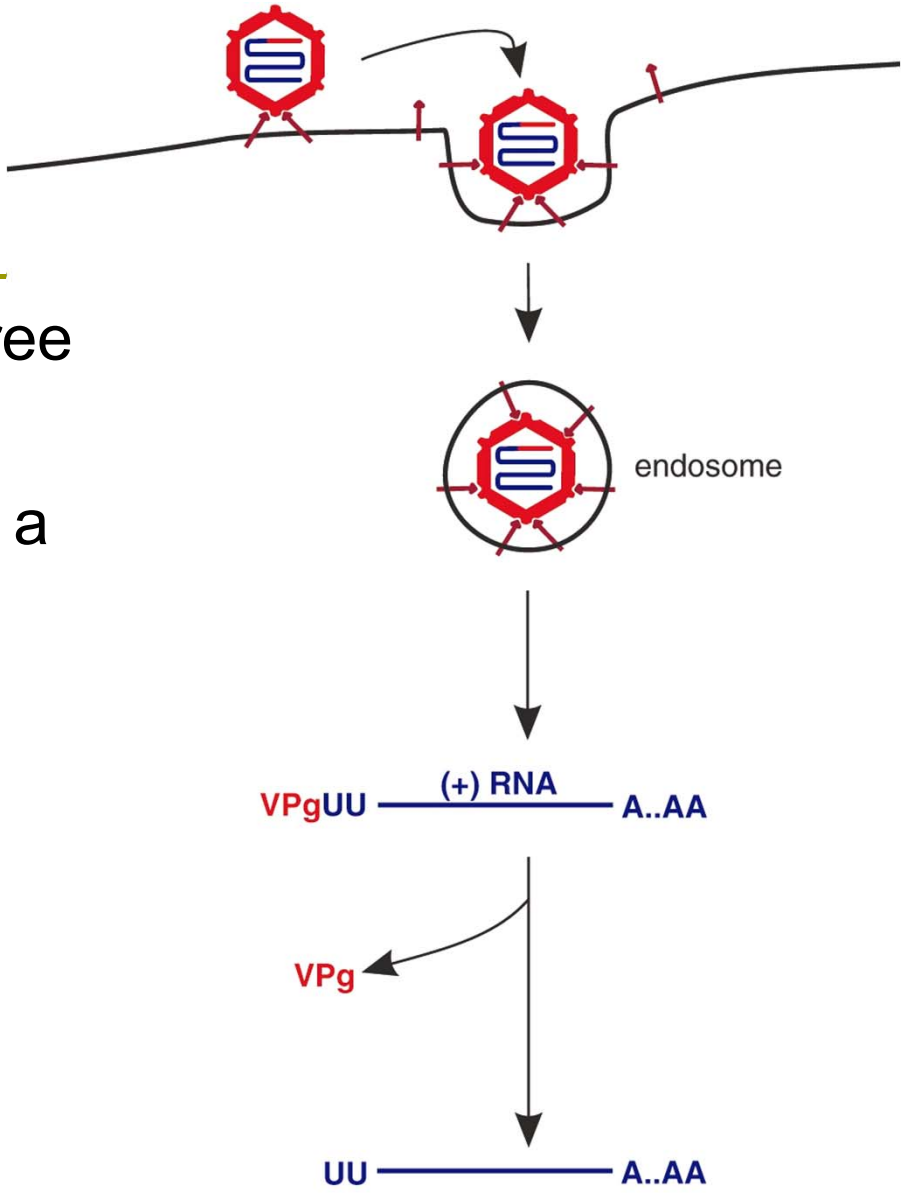
- Binding of a virion to receptors results in major changes to the capsid structure:
  - ✓ the N termini of VP1 move from the interior to the exterior surface of the capsid
  - ✓ VP4 is lost from the virion



14.4.2 Entry

- Several modes of genome entry into the host cell have been proposed for picornaviruses:
  - ✓ One mode involves transfer of the RNA from the virion into the cytoplasm at the plasma membrane, leaving the capsid at the cell surface.
  - ✓ Other modes involve endocytosis, followed by either release from the capsid after disruption of the endosome membrane, or release of RNA from the capsid then transport across the endosome membrane.

- Once the virus genome is free in the cytoplasm the VPg is removed from the 5’ end by a cell enzyme.

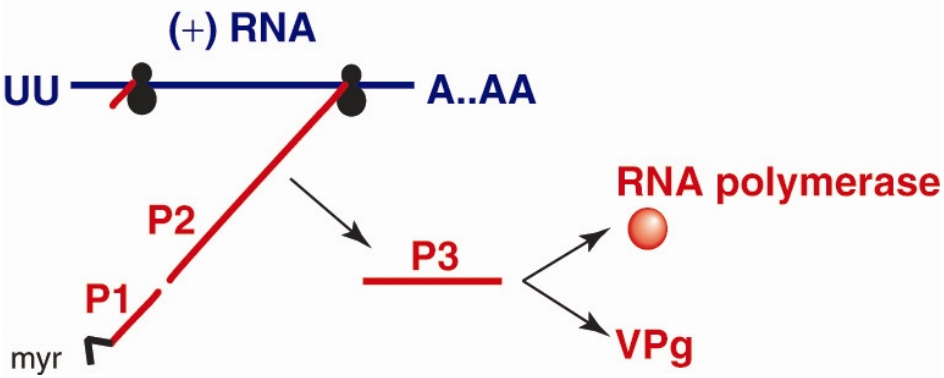


Entry of picornavirus virion by endocytosis

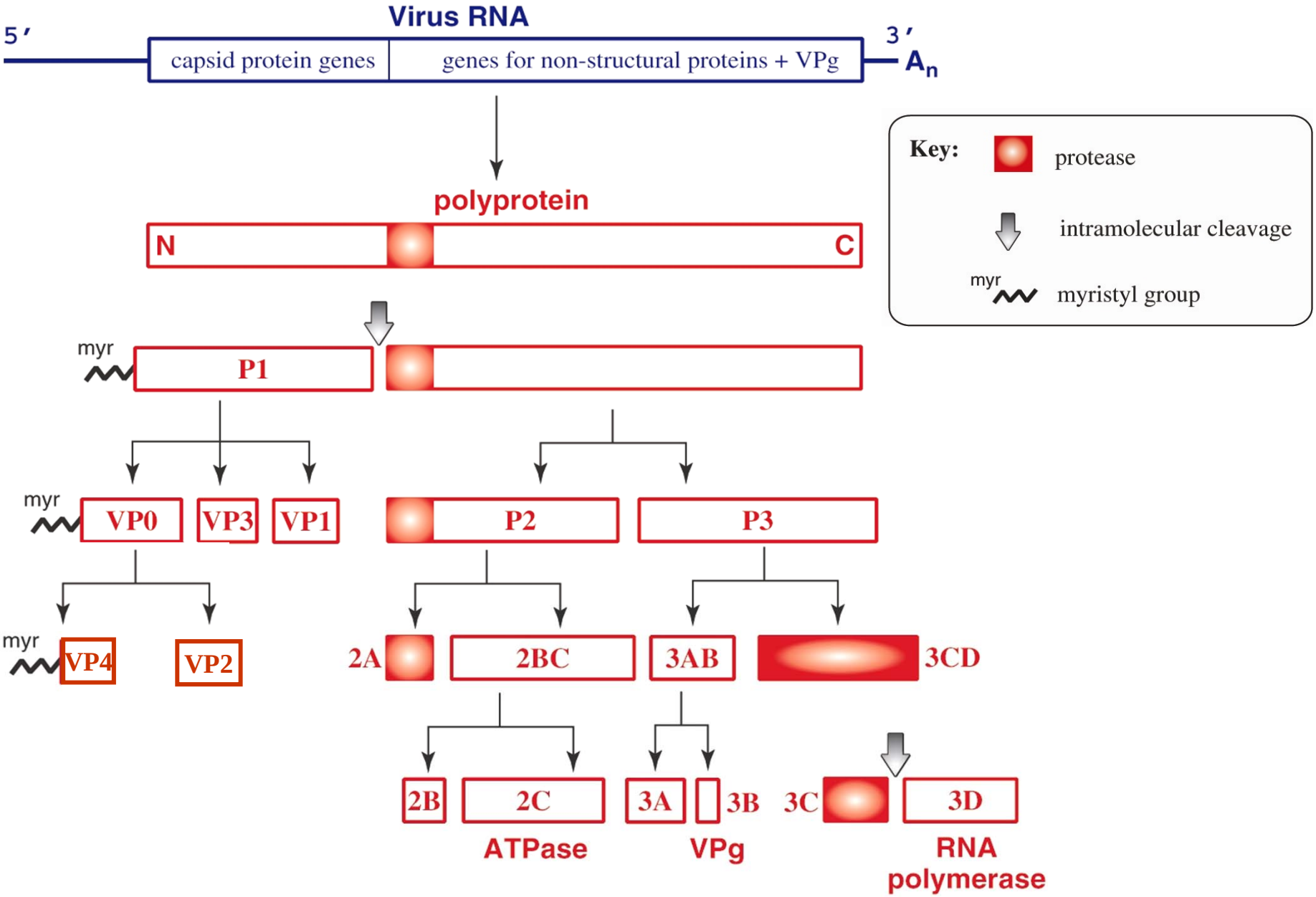
14.4.3 Translation and post-translational modifications

- The virus genome functions as mRNA, but as the RNA is not capped, the preliminary stages of translation are different to those for capped mRNAs.
  - ✓ The 40S ribosomal subunit binds not at the 5' end of the RNA, but internally at the IRES.
  - ✓ Most of the eukaryotic initiation factors (eIFs, normally involved in cap-dependent translation) are involved, but a notable exception is the cap-binding protein eIF4E.

- The genome encodes a single polyprotein, which undergoes a series of cleavages to give rise to all the structural and non-structural proteins.



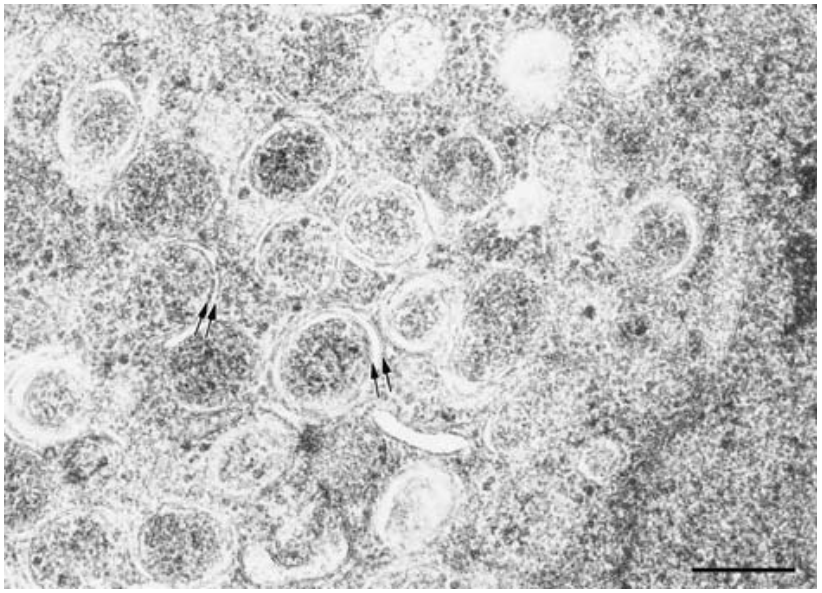
- A polyprotein is synthesized and rapidly cleaved into P1, P2 and P3.
- The N terminus of P1 is myristylated.
- Further proteolytic cleavages take place producing a number of proteins.


Virus RNA
5′
capsid protein genes
genes for non-structural proteins + VPg
3′
An
polyprotein
N
C
Key:
protease
intramolecular cleavage
myr
myristyl group
myr
P1
myr
VP0
VP3
VP1
P2
P3
myr
VP4
VP2
2A
2BC
3AB
3CD
2B
2C
3A
3B
3C
3D
ATPase
VPg
RNA polymerase

- The production of the proteins via a polyprotein precursor might suggest that equimolar amounts result, but not all cleavages take place with equal efficiency.
  - ✓ Ex: greater amounts of the capsid proteins are produced than of 3D (the RNA polymerase).

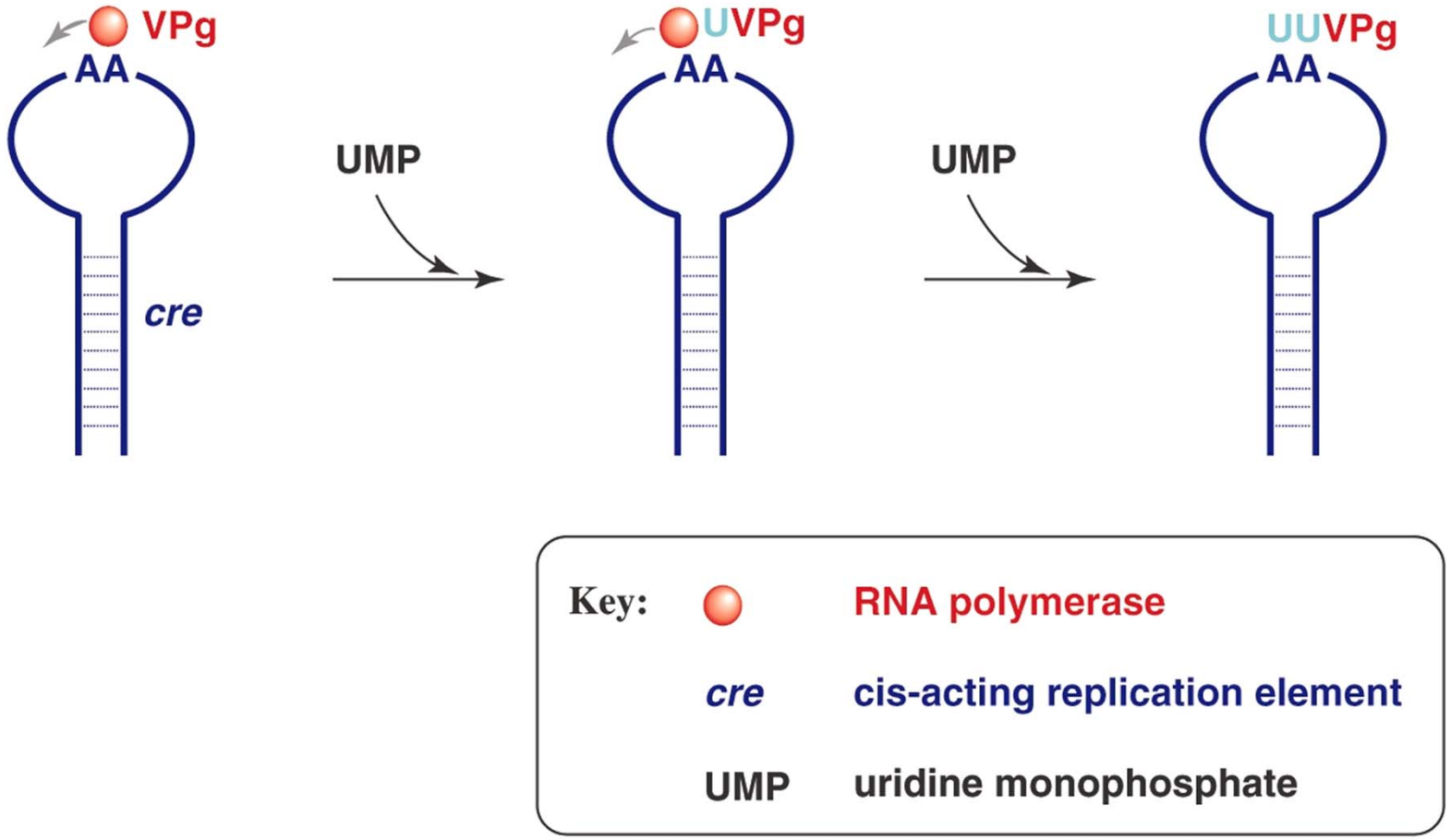
14.4.4 Transcription/genome replication

- RNA synthesis occurs in replication complexes that contain cell proteins, as well as virus proteins and RNA.
  - ✓ Using immune electron microscopy it has been shown that the replication complexes are associated with the cytoplasmic surfaces of membranous vesicles that develop in the cytoplasm of infected cells.

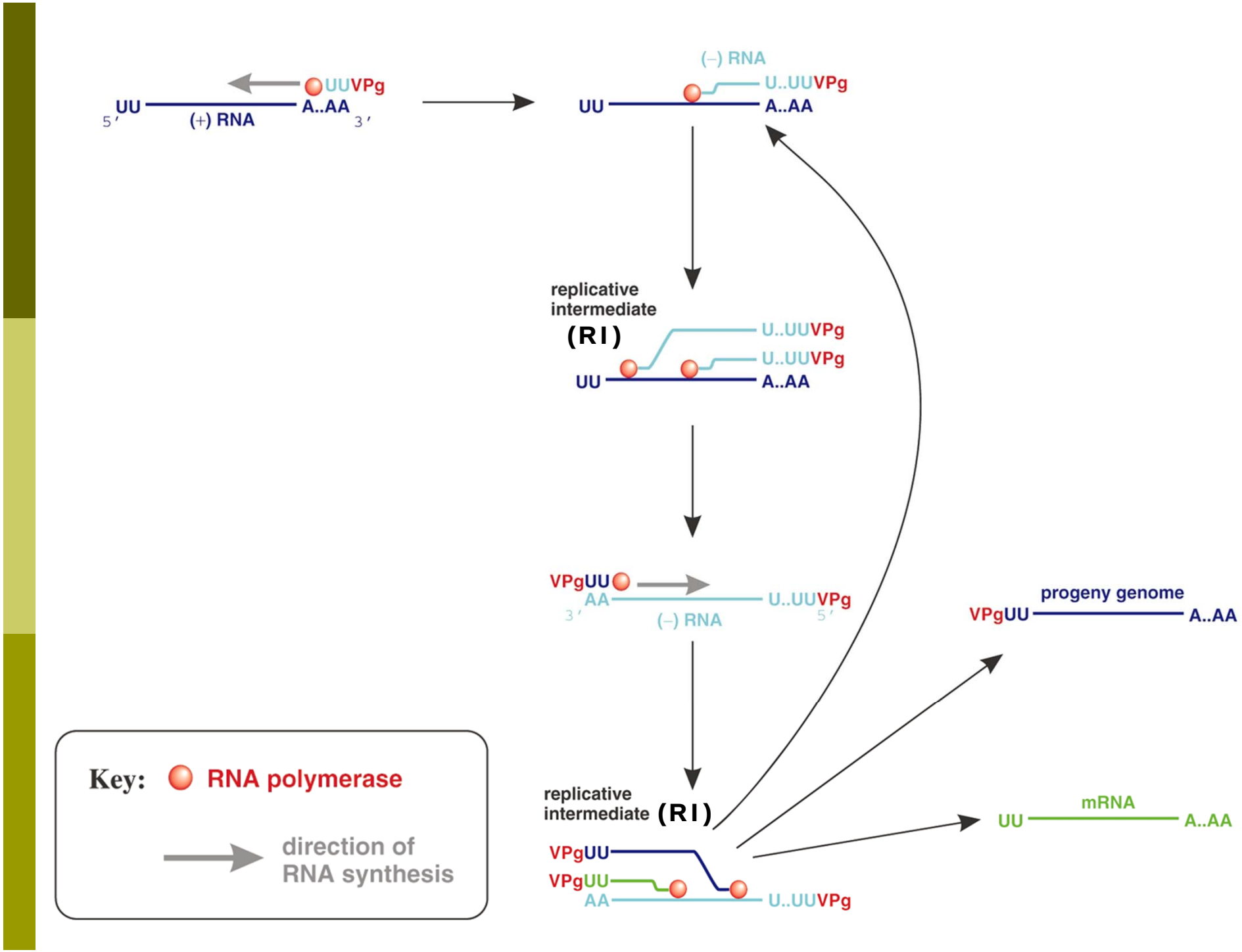


- In order for the infecting (+) RNA to be replicated, multiple copies of (−) RNA must be transcribed and then used as templates for (+) RNA synthesis.
- The primer for both (+) and (−) strand synthesis is the small protein VPg, which is uridylylated at the hydroxyl group of a tyrosine residue by the poliovirus RNA polymerase.
  - ✓ These reactions take place at a cis-acting replication element (cre) located in a stem-loop in the virus genome; an AA sequence within the loop forms the template for UU synthesis.

Uridylylation of poliovirus VPg



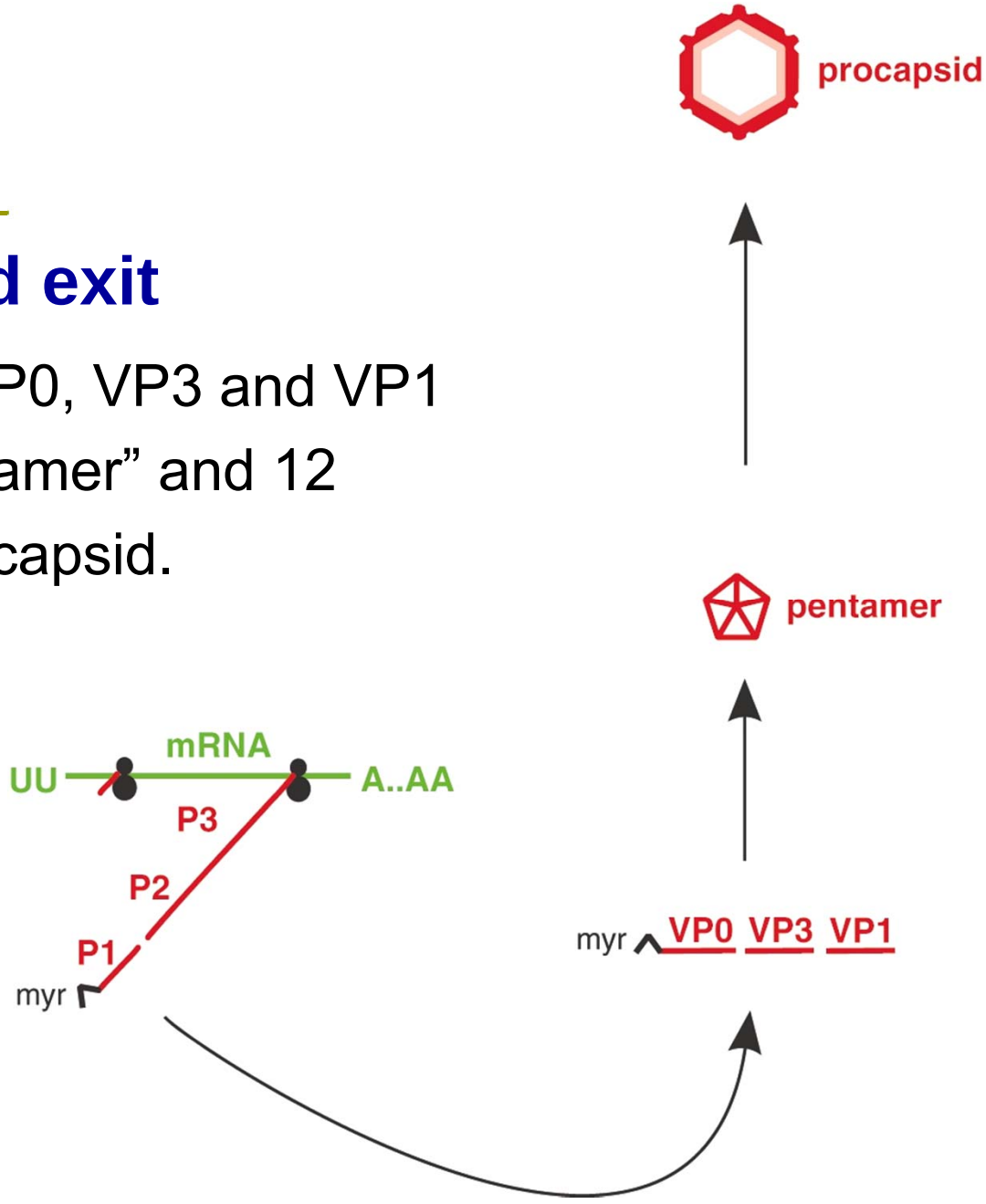
- Much of the RNA in the replication complexes is present as replicative intermediates (RIs).
  - ✓ An RI is an association of RNA molecules consisting of a template RNA and several growing RNAs of varying length.
  - ✓ Thus, some RIs consist of a (+) RNA associated with growing (−) RNAs, while other RIs consist of a (−) RNA associated with growing (+) RNAs.
  - ✓ Also associated with RIs are copies of the RNA polymerase.


UUVPg
UU
A..AA
5′
(+) RNA
3′
(–) RNA
U..UUVPg
UU
A..AA
replicative
intermediate
(RI)
U..UUVPg
U..UUVPg
UU
A..AA
VPgUU
AA
3′
U..UUVPg
(–) RNA
5′
progeny genome
VPgUU
A..AA
replicative
intermediate (RI)
mRNA
UU
A..AA
VPgUU
VPgUU
AA
U..UUVPg
Key:
RNA polymerase
direction of
RNA synthesis

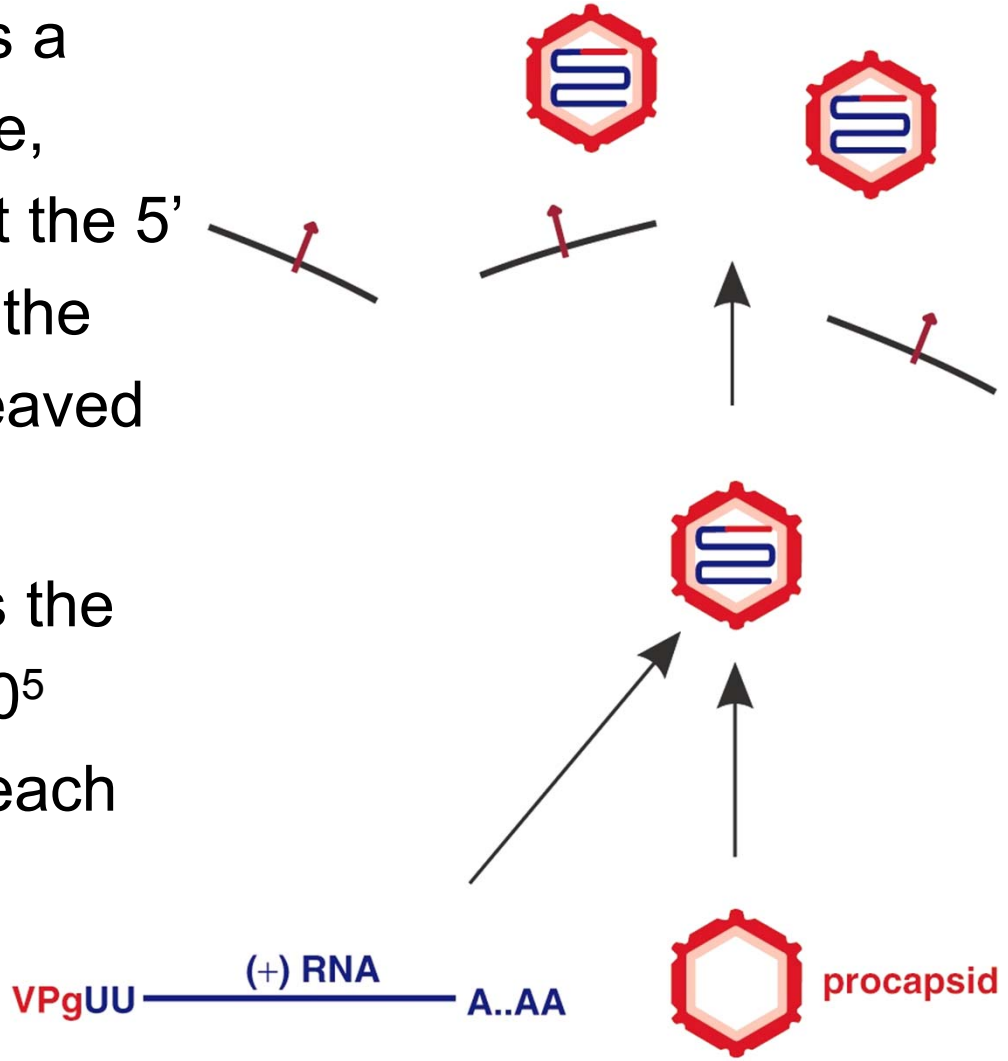
- It has been estimated that in a poliovirus-infected cell up to 2500 RNA molecules are synthesized per minute.
- The virus controls RNA synthesis in such a way that about 50 times more (+) RNA than (−) RNA is made. Some of the (+) RNA molecules are used as:
  - ✓ templates for further (−) RNA synthesis
  - ✓ mRNA
  - ✓ the genomes of progeny virions

14.4.5 Assembly and exit

- Five copies each of VP0, VP3 and VP1 assemble into a “pentamer” and 12 pentamers form a procapsid.

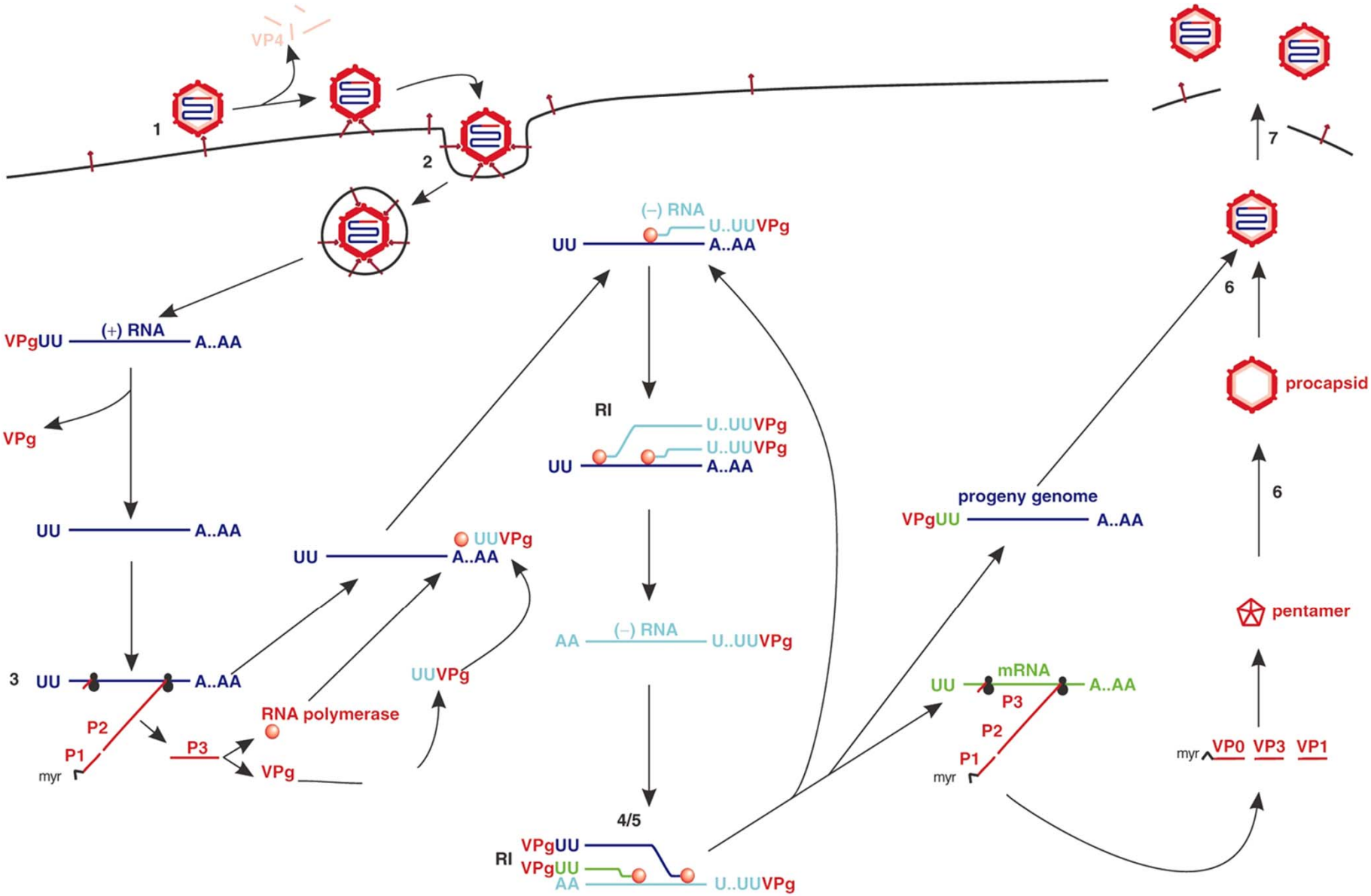


- Each procapsid acquires a copy of the virus genome, with VPg still attached at the 5' end. At around this time the 60 copies of VP0 are cleaved into VP4 and VP2.
- Lysis of the cell releases the virions; approximately 10^5 virions are produced in each poliovirus-infected cell.



14.4.6 Inhibition of host gene expression

- Soon after infection, the expression of host cell genes is inhibited.
  - ✓ In poliovirus-infected cells, it has been demonstrated that all three host RNA polymerases are rapidly inhibited, thus terminating transcription.
- Translation from pre-existing cell mRNAs is inhibited as a result of the cleavage of a translation initiation factor by the virus protease 2A.
  - ✓ Virus protein synthesis is unaffected as it is initiated by a cap-independent mechanism.



1. Attachment

2. Entry

3. Translation

4/5. Transcription/Genome replication

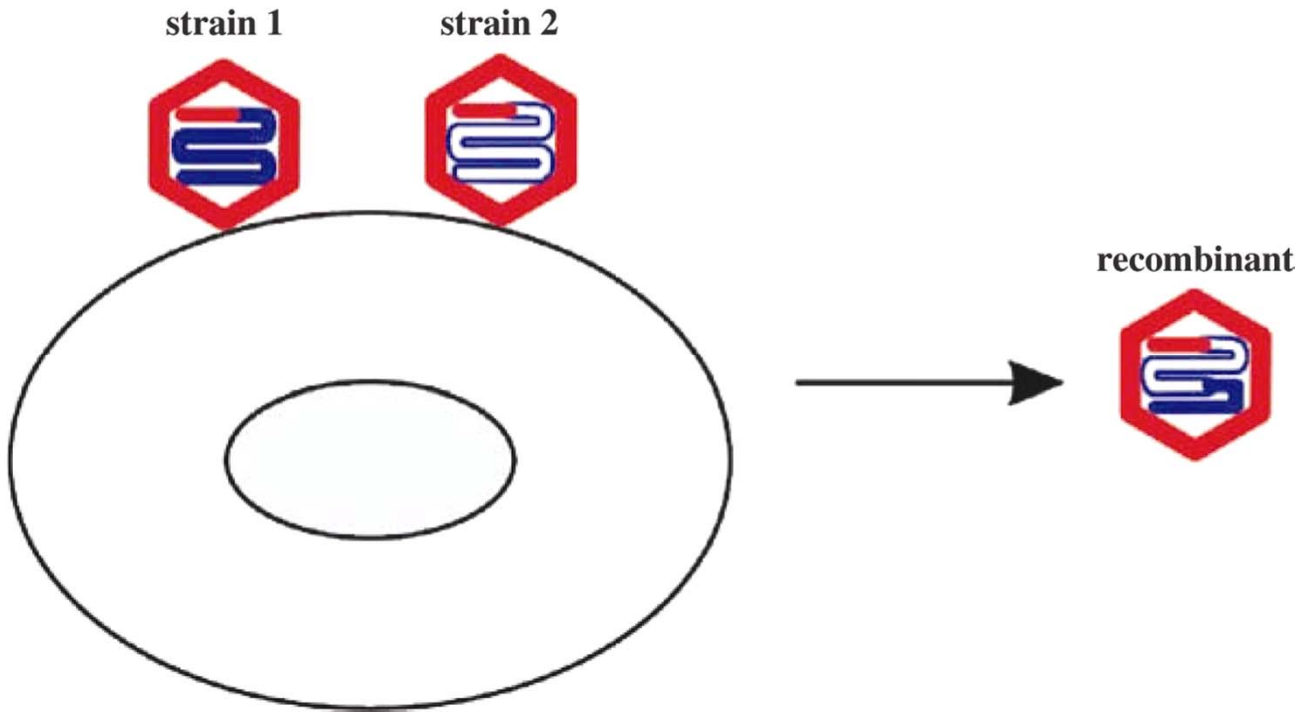
6. Assembly

7. Exit

- The following points should be noted for class IV viruses (ssRNA, +) in general:
  - ✓ Translation occurs before transcription.
  - ✓ RNA replication takes place on membranes.
  - ✓ The processes of transcription and genome replication are one and the same, resulting in the production of (+) RNA.
  - ✓ (+) RNA has three functions:
    - templates for (–) RNA synthesis
    - mRNA
    - genomes of progeny virions

14.5 Picornavirus recombination

- If a cell becomes infected with two strains of a picornavirus, then recombinant viruses may be present amongst the progeny virus from that cell.

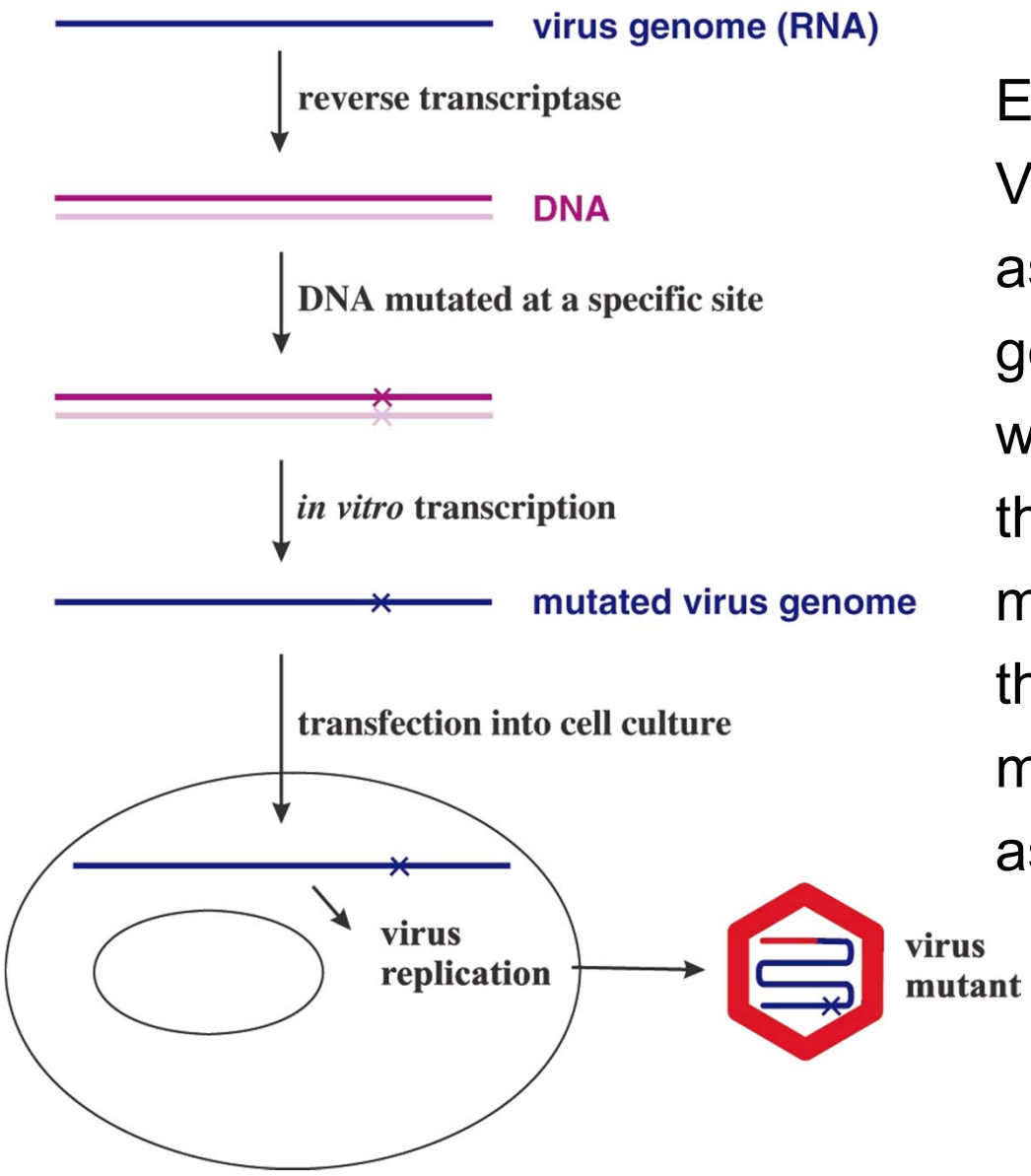


- The oral polio vaccine contains attenuated strains of all three poliovirus serotypes.
  - ✓ Administration of this vaccine can lead to co-infections of gut cells with two serotypes, and recombinants between serotypes may be formed.
  - ✓ If a wild type poliovirus is also present in the gut then recombination may occur between the wild type virus and one of the vaccine strains. There have been a number of documented cases where recombinants have caused polio.

14.6 Picornavirus experimental systems

14.6.1 Reverse genetics

- Techniques that have been developed for manipulating DNA genomes can be applied to RNA genomes if the RNA is reverse transcribed to DNA.
  1. A defined mutation can be introduced into a gene in the DNA to investigate the function of the gene.
  2. After mutation the DNA is transcribed back to RNA, which is then transfected into cells in culture.
  3. Virus replication in the transfected cells leads to the production of mutant virus.

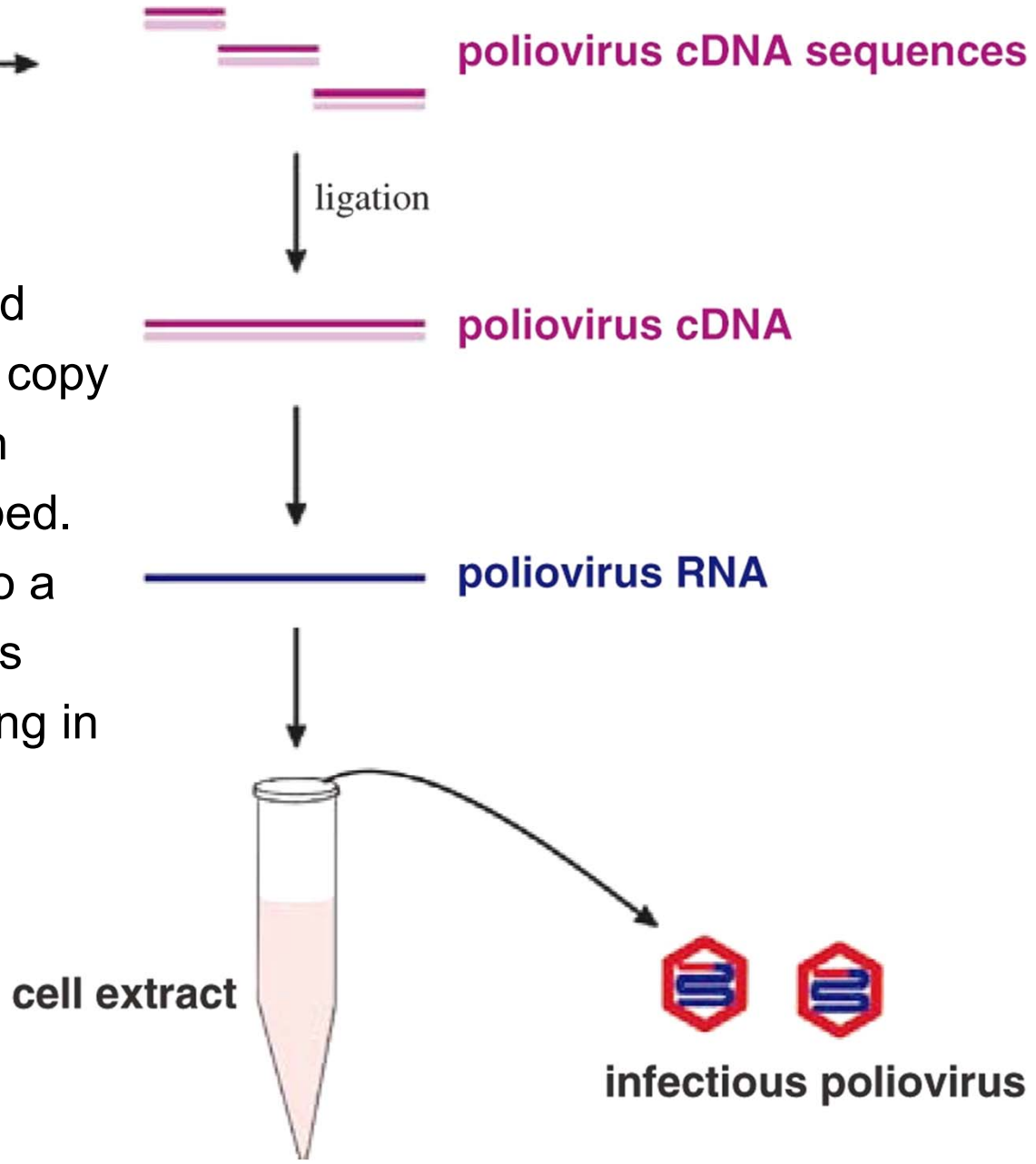


Ex: myristylation of poliovirus VP0 is essential for virion assembly to proceed. A genome was produced in which the glycine residue at the N terminus of VP0 was mutated to alanine; VP0 of the mutant virus could not be myristylated and virion assembly was inhibited.

14.6.2 Cell-free synthesis of infectious virus

- In 1991 a group of workers recovered infectious poliovirus from a HeLa cell extract to which they had added purified poliovirus RNA.
- Cell-free systems based on cytoplasmic extracts have subsequently been used to investigate features of the poliovirus replication cycle that are impossible to dissect using cell-based systems.
- In 2002, infectious poliovirus had been produced in a process that started with the synthesis of a copy DNA (cDNA) of the virus genome.

Short poliovirus cDNA sequences are synthesized and ligated to form a DNA copy of the genome, from which poliovirus RNA is transcribed. The RNA is introduced into a cell extract, where it directs virus replication, culminating in the assembly of infectious virus.



14.7 Other plus-strand RNA viruses

- The genomes of plus-strand RNA viruses are translated in the infected cell prior to their transcription. Because the ribosomes in eukaryotic cells usually terminate after translation of the first ORF, the viruses need strategies to ensure that all the proteins encoded in the infecting genome are translated.
  1. Polyprotein
  2. Subgenomic mRNAs
  3. Segmented genome

1. Polyprotein
2. Subgenomic mRNAs: The genome has two or more ORFs. The RNA-dependent RNA polymerase is encoded by the ORF at the 5’ end of the genome so that it can be translated from the infecting genome. The other ORF(s) are transcribed into subgenomic mRNA(s) that have the same 3’ end as the genome.
3. Segmented genome: There is one ORF in each RNA segment.

	Family	Virus example(s)	Coding strategy
Animal viruses	*Flaviviridae*	West Nile virus (Section 21.3.1) Hepatitis C virus (Section 22.7)	Polyprotein
	Coronaviridae	Severe acute respiratory syndrome (SARS) virus (Section 21.5.1)	Subgenomic mRNAs
	Togaviridae	Rubella virus	Polyprotein plus subgenomic mRNAs
Plant viruses	*Potyviridae*	Potato virus Y	Polyprotein
	Flexiviridae	Potato virus X	Subgenomic mRNAs
	Comoviridae	Cowpea mosaic virus	Polyprotein plus segmented genome

補充：感染水產生物的單股正向RNA病毒

- 正向單股RNA病毒：
  - ✓ 為動物病毒中構造最小的最簡單的，也是演化上最原始的病毒之一，大小上差異很大。
  - ✓ 此類病毒分為無外套膜及有外套膜兩種，無外套膜之病毒呈二十面體，有外套膜之病毒則為圓球型。
  - ✓ 共分為八科及一獨立屬，其中與水產生物較相關的有三科：
    - 小RNA病毒科 (*Picornaviridae*)
    - 披膜病毒科 (*Togaviridae*)
    - 野田病毒科 (*Nodaviridae*)

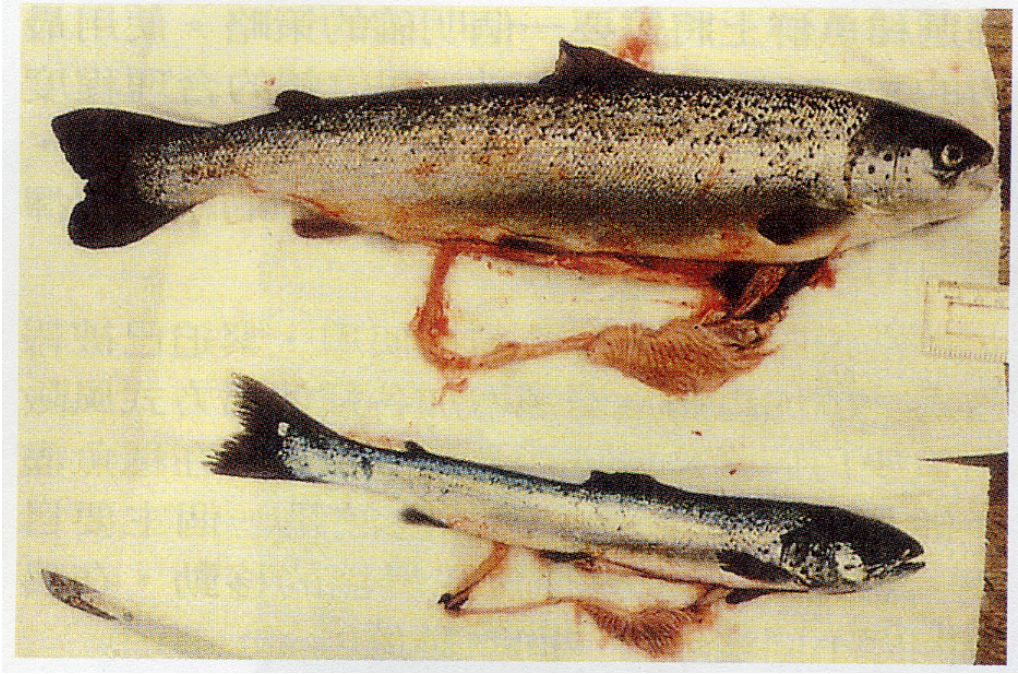
小RNA病毒科 (*Picornaviridae*)

- 病毒顆粒呈圓球型，以電子顯微鏡可觀察到病毒二十面體的結構。
- 直徑30nm，分子量8~9 x 10^6Da，無外套膜與spike，對乙醚、氯仿、酸性環境等具有抗性，各種病毒對熱的耐受性不盡相同。
- 本科共分為五屬，約200種以上的病毒。
- 此科病毒在魚類的報告有限，目前發現的有香魚與鮭魚的小RNA病毒，但在基因與蛋白質序列上的瞭解並不多。

- 披膜病毒科 (*Togaviridae*)
  - 此科病毒分為兩屬：阿爾伐病毒屬 (*Alphavirus*) 與風疹病毒屬 (*Rubivirus*)。
  - 阿爾伐病毒屬的病毒成圓球型，風疹病毒屬則為多態型，分子量52 × 10^6Da，對乙醚、氯仿及清潔劑極敏感。
  - 披膜病毒所引起的魚類疾病有兩種，分別為1984年Munro在蘇格蘭養殖的大西洋鮭魚中發現的胰臟病 (Pancreas disease, PD)，與1992年Holt與Rohovec在美國哥倫比亞銀鮭中發現的紅血球內包涵體症 (Erythrocytic inclusion body syndrome, EIBS)。

胰臟病，PD

- 病魚精神沈鬱，食慾不佳，常躲於籠子角落，主要特徵為背部顏色變深，除此之外沒有其餘肉眼可見的症狀，解剖後可見盲腸間的胰臟組織變紅，急性階段可觀察到鄰近體腔有輕微的出血，疾病爆發後約3個月左右，可看到魚體的大小相差很多。



野田病毒科 (*Nodaviridae*)

- 係由病毒發現地為日本野田村 (Nodamura)而命名，共有七屬。
- 病毒顆粒為二十面體，直徑30nm，分子量8×10^6Da，無外套膜，耐酸、對氯仿具有抗性。
- 魚類的nodavirus最先於1988年時，由Balance與Gallet在海水養殖的鱸魚身上發現，之後被命名為病毒性神經壞死病 (Viral nervous necrosis, VNN)，目前已有19個魚種曾診斷出感染此病毒。